The Mouse Skin Model Is Used To Assess Relative Tumorigenic Potential

- Scientific Community consensus (40 years) supports the use of the chronic mouse skin model as an assessment of the tumorigenic potential of cigarette smoke condensate.
- Substantial, statistically-significant and reproducible tumorigenic responses have been observed in mouse dermal-application studies with cigarette smoke condensate.
- The NCI used the Mouse Skin Model for the a ten year program to identify changes "Towards a Less Hazardous Cigarette"

Tumor Promotion Assay Provides Similar Data in less Time

- Multi-stage tumorigenicity (initiation/promotion) model is used.
- Tumor initiation potential is indicated by in vitro assays.
- Tumor promotion assays in mouse skin provide rapid, repeatable, dose-dependent tumor development responses to cigarette smoke condensate exposure.
- Substantial, statistically-significant responses provide a means to compare tumor promotion activity and measure progress toward risk reduction.

Study Protocols Selected for Toxicology Assessments of Cigarettes

Subchronic Inhalation Studies

- Sprague-Dawley Rat - R. J. Reynolds Tobacco (ECLIPSE 7-026)
- Syrian Golden Hamster - Battelle Northwest Laboratories (ECLIPSE 5-014)

Dermal Tumor Promotion Studies

- Sencar Mice - R. J. Reynolds Tobacco (ECLIPSE 7-026)

SUBCHRONIC NOSE ONLY INHALATION STUDIES

Subchronic Inhalation Studies Comparative Approach

- Smoke Related Effects identified by comparison to controls
- Smoke Related Effects compared at equal exposure concentrations

Subchronic Inhalation Studies Exposure Conditions

- Rats and hamsters are exposed nose-only 1 hour per day, 5 days per week, during a 13 week period.
- Concentrations of WTPM used were:
  - 0.00 mg WTPM/L - SHAM exposure
  - 0.16 mg WTPM/L - LOW exposure
  - 0.32 mg WTPM/L - MID exposure
  - 0.64 mg WTPM/L - HIGH exposure
- FTC conditions

Source: https://www.industrydocuments.ucsf.edu/docs/rfdk0001

ANIMAL TOXICOLOGY STUDIES FOR ASSESSING RISK REDUCTION POTENTIAL

OECD Guidelines Considerations for Evaluation of Health Effects

- Harmonized approach assures wide acceptability of test data.
- Scientific judgment must be used on a case by case basis concerning the suitability of a particular test method.
- Animal testing is defined by the route of human exposure.

Evaluation of Potential Toxicity A Comparative Approach is Necessary

- Cigarettes possess a widely recognized background level of toxicity in animal models.
- A comparative approach is essential to measure progress toward minimizing risk of a cigarette.
- Equal exposures of "Reference" cigarettes are compared to "Test" cigarettes to assess the impact of specific changes relative to background level of toxicity.
- All Animal Toxicology Assays conducted by RJRT use a comparative approach.

Summary of Rationale for 90-Day Subchronic Inhalation Study Using the Rat

- Study designs are consistent with FDA-GLP and OECD Guidelines
- Acute and short-term studies, recommended by OECD, are not useful for smoke.
- Subchronic Study provides all short-term data and all additional, necessary pathology data.
- Chronic studies provide no additional information beyond the changes observed at 90 days.

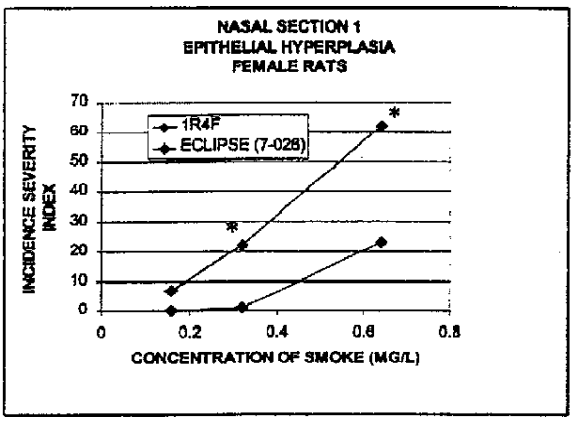
Summary of Rationale for 90-Day Subchronic Inhalation Study Using the Rat

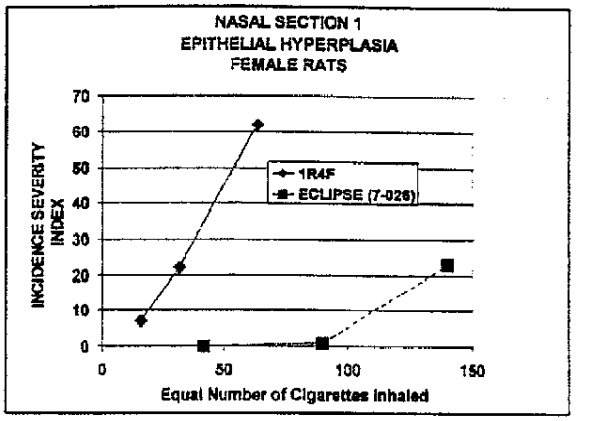
- The subchronic inhalation study exposure is sufficient, and duration is long enough, to identify all toxic effects.
- The subchronic inhalation study will detect new toxicities of cigarette smoke, if introduced.
- This study design elicits substantial responses sufficient to compare differences in cigarette toxicities.

Smoke Tumorigenesis Can Not be Assessed Using the Inhalation Model

- Studies designed to reproduce the observations of respiratory tract cancer have met with little or no success (Dalbey et al.)
- Lack of reproducibility and weak statistical power limit discrimination of differences even between treated and untreated animals.
- Thus, chronic inhalation studies do not form a sound basis to compare the potential tumorigenic effects of different cigarettes.

Source: https://www.industrydocuments.ucsf.edu/docs/rfdk0001





Subchronic Nose-Only Inhalation Studies
Rat Study

Smoke-Related Changes: Eclipse Relative to 1R4F

Histopathology Observations	7-026A	% 1R4F
Nasal 1		
Chronic/active Inflammation	Reduced	75
Epithelial hyperplasia	Reduced	23
Larynx: Chronic/active inflammation	Similar	98
Ventral Larynx:		
Squamous metaplasia	Reduced	75
Acute inflammation	Similar	82
Trachea: Epithelial hyperplasia	Reduced	74
Lung		
Goblet cells	Not a call	96
Non-pigmented macrophages	Similar	90
Individual B/G pigmented macrophages	Absent	0
Aggregates of B/G pigmented macrophages	Absent	0

Subchronic Nose-Only Inhalation Studies
Hamster Study

Smoke-Related Changes: Eclipse Relative to 1R4F

Histopathology Observations	5-535	% 1R4F
Nasal 1		
Epithelial hyperplasia	Reduced	7
Larynx, dorsolateral walls		
Squamous metaplasia	Reduced	7
Larynx, ventral pouch		
Hyperplasia	Reduced	22
Larynx, protuberance		
Suppurative inflammation	Reduced	6
Hyperkeratosis	Reduced	12
Hyperplasia	Reduced	38
Squamous metaplasia	Reduced	38
Larynx, epiglottis		
Hyperplasia	Reduced	9
Squamous metaplasia	Reduced	10

Subchronic Inhalation Assay Summary

- The Subchronic Inhalation Assay is sensitive to changes in tobacco smoke.
- The assay clearly differentiated ECLIPSE's simpler smoke from the smoke of a marketplace reference cigarette.
- The reduced toxicity is compatible with the potential to present less risk for smokers.

30-WEEK DERMAL TUMOR PROMOTION ASSAY IN THE SENCAR MOUSE

Source: https://www.industrydocuments.ucsf.edu/docs/rfdk0001

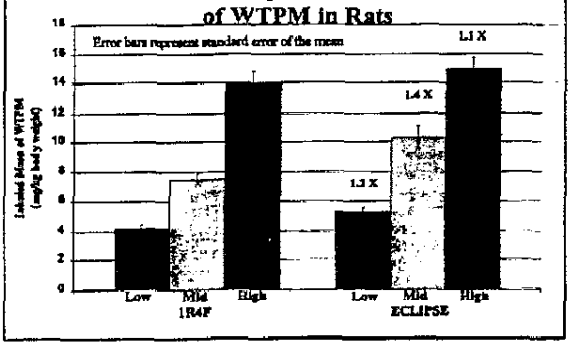
Subchronic Inhalation Studies
Biological Endpoints

- In-life observations
  - Respiratory Patterns
  - Clinical observations
  - Body weights
- Pathology
  - Gross changes
  - Organ weights
  - Organ weight ratios
  - Histopathology

Subchronic Inhalation Studies
Tissues Evaluated

Adrenals	Aorta	Bone (marrow: femur)
Bone (sternum)	Brain	Cecum
Colon	Cranium	Duodenum
Epididymides	Esophagus	Eyes/optic nerve
Heart	Ileum	Jejunum
Kidneys	Larynx	Liver
Lung	Lymph Nodes	Mammary glands
Nasopharynx	Nose/turbinates	Ovaries
Pancreas	Parathyroid	Pituitary
Prostate	Rectum	Salivary gland
Seminal vesicle	Skeletal muscle	Skin (abdominal)
Spinal Cord (lumbar)	Spleen	Stomach
Tail	Testes	Thymus
Thyroid	Trachea	Urinary Bladder
Uterus	Zymbal's gland	

Subchronic Inhalation Studies
Measure of Exposure Inhaled Mass of WTPM in Rats



Subchronic Inhalation Studies
Clinical Observations

- No significant clinical observations were observed in either RATS or HAMSTERS for either the ECLIPSE or 1R4F cigarette.

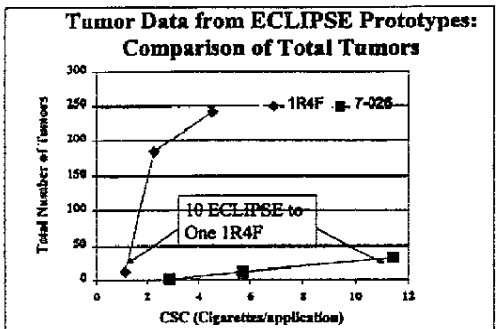
Subchronic Inhalation Studies
Findings at Necropsy - Rats and Hamsters

- No biologically significant findings:
  - Gross observations
  - Organ weights
  - Organ weight ratios

Subchronic Inhalation Studies
Comparisons of Histological Findings

- All Smoke-related changes compared:
  - Low ECLIPSE compared to low 1R4F
  - Mid ECLIPSE compared to mid 1R4F
  - High ECLIPSE compared to high 1R4F
- Kolmogorov-Smirnov test - incidence and degree of change are compared between groups; $p < 0.05$

Source: https://www.industrydocuments.ucsf.edu/docs/rfdk0001



Percent Reductions in Tumorigenicity Parameters for ECLIPSE

	Tumor-Bearing Animals			Total Tumors		
	1R4F	7-026	% Reduction	1R4F	7-026	% Reduction
Low	6	1	83	11	1	91
Mid	23	2	91	184	11	94
High	27	12	56	242	31	87

Tumorigenesis Summary

- The Dermal Tumor Promotion Assay is sensitive to changes in tobacco smoke condensate.
- The assay clearly differentiated a simpler smoke from the smoke of a marketplace surrogate cigarette.
- The reduced tumorigenicity is compatible with the potential to present less risk for smokers.

ANIMAL TOXICOLOGY CONCLUSIONS

The Subchronic Inhalation Assay and the Dermal Tumor Promotion Assay were sensitive to changes in smoke.

Significant profiles of reduced biological activities from ECLIPSE's simpler smoke were observed.

The profiles of reduced biological activities are all compatible with the potential to present less risk for smokers.

Source: https://www.industrydocuments.ucsf.edu/docs/rfdk0001

Dermal Promotion Study
Methods

- Eight groups of 40 female SENCAR mice
- 10, 20 or 40 mg "tar" / application
- Dosing three times / week, 200 ul / application
- Clipped once per week

Dermal Promotion Study
Experimental Control Groups

Group Number	Treatment (initiator / promoter)
1	Acetone / Acetone
2	DMBA / Acetone

Dermal Promotion Study
Experimental Groups

Group Number	Treatment (initiator/promoter)		
3	DMBA / 1R4F	CSC	10 mg "tar"/appl
4	DMBA / 1R4F	CSC	20 mg "tar"/appl
5	DMBA / 1R4F	CSC	40 mg "tar"/appl
6	DMBA / ECLIPSE	CSC	10 mg "tar"/appl
7	DMBA / ECLIPSE	CSC	20 mg "tar"/appl
8	DMBA / ECLIPSE	CSC	40 mg "tar"/appl

Dermal Promotion Study
Biological End Points

Body Weights

Clinical Observations

Tumor Tracking

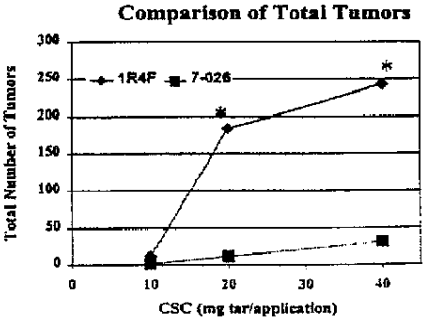
Organ Weights

Histopathology

Dermal Promotion Study
Results - Biological Endpoints

- Survival - no significant findings
- Body weights - no significant findings
- Clinical Signs of Toxicity:
  - ECLIPSE - no significant findings
  - 1R4F - Skin Irritation
- Organ Weights - no significant findings

Tumor Data from ECLIPSE Prototypes: Comparison of Total Tumors



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